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Effect of calprotectin subunit S100A9 on the expression and methylation of *OCLN* in human melanoma cell line A-375

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Abstract: Increased levels of calprotectin subunits S100A8 and S100A9 have been detected in human cancers. Melanoma is the most aggressive type of skin cancer, and its treatment is challenging because of its brain metastasis. *OCLN* encodes occludin, which plays a major role in the formation and regulation of tight junctions. The aim of this study was to evaluate the methylation status of the *OCLN* promoter and its expression in A-375 melanoma cells treated with or without various concentrations of S100A9 for 24, 48, and 72 h. Total RNA was extracted, and synthesized cDNA was assessed by performing real-time PCR. MSP-PCR was performed after treatment with bisulfite. Recombinant S100A9 inhibited the proliferation of the A-375 cell line and the expression of the *OCLN* gene was downregulated in a time- and concentration-dependent manner. Results of MSP-PCR showed that the *OCLN* gene promoter in a human melanoma cell line (A-375) was semimethylated.

Key words: Melanoma, *OCLN*, calprotectin subunit S100A9, methylation, promoter

1. Introduction

The S100 protein family includes >20 low-molecular-weight intracellular proteins containing an EF-hand motif and showing calcium-binding properties (Donato, 2001). A recent study showed that S100 proteins play important roles in cancer malignancy and metastasis (Donato, 2003). Most S100 proteins perform several intracellular and extracellular functions. Intracellular functions of S100 proteins include regulation of calcium homeostasis, cell cycle, cell growth, and cell migration and regulation and phosphorylation of transcriptional factors. Extracellular function of S100 proteins is activated by the binding of tumor necrosis α (TNF-a), interleukin-1 (IL-1), IL-6, chemokines, matrix metalloproteinases (MMPs), angiogenesis factors, and antiapoptotic proteins as cytokines to cell surface receptors (Chen et al., 2014). Three receptor types recognize S100 proteins, namely the receptor for advanced glycation end products (RAGE), Toll-like receptors (TLRs), and the extracellular MMP inducer (EMMPRIN) (Hiratsuka et al., 2008). RAGE is a multiligand receptor belonging to the immunoglobulin superfamily that is involved in inflammation, diabetes, atherosclerosis, nephropathy, neurodegeneration, and cancer (Sorci et al., 2004). S100A9, a member of the S100 protein family, interacts with RAGE, TLRs, and EMMPRIN (Sakaguchi et al., 2011). EMMPRIN is a well-known cell surface molecule associated with cancer cell malignancy (Gabison et al., 2005). The binding of S100A9 to EMMPRIN provides new insight into the molecular mechanism underlying S100A9–EMMPRIN-mediated inflammation and melanoma cancer cell metastasis (Sakaguchi et al., 2011). EMMPRIN is also involved in fetal development, retinal function, nervous system development, and plaque formation in Alzheimer disease (Iacono et al., 2007). S100A9 is mainly expressed in myeloid cells and performs a key role in these cells under inflammatory conditions (Urban et al., 2009). In humans, serum levels of S100A9 increase during several disease conditions, including acute inflammatory lesions, cystic fibrosis, rheumatoid arthritis, and amyloids in the aging prostate gland (Vogl et al., 2012). Melanoma is the most aggressive type of skin cancer, involving a type

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